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RESEARCH ARTICLE

Identifying Potential Donor Parents for Breeding Against Leaf Curl Virus and Anthracnose Diseases in Chilli

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Abstract

Development of multiple disease-tolerant chilli (*Capsicum annuum* L.) varieties, which can be grown successfully throughout the year, is a goal of plant breeders. The study was conducted to identify chilli genotypes that could be utilized in hybridization to retrieve potential genotypes having tolerance to both chilli leaf curl virus (ChiLCuV) and chilli anthracnose diseases in addition to higher yield. About 26 chilli genotypes were screened for tolerance to ChiLCuV and chilli anthracnose diseases under field and laboratory conditions, and for other quantitative traits. A wide range of genetic variation was observed for all traits under study. Genotypes were grouped into 6 clusters that do not represent the same place of origin, indicated genotypes in a cluster were geographically diverse and genotypes obtained from the same region were genetically different. The principal components, percent disease index (PDI) of ChiLCuV, PDI of chilli anthracnose, and plant height, had eigenvalue >1 and together accounted for almost 100% of the variation. Based on multivariate analysis, fruit yield and tolerance to both ChiLCuV and chilli anthracnose diseases, genotypes 'Bidhan Chilli 4', 'Pant C 1', and 'Chinese Bona' were identified as tolerance sources against both diseases. Highly tolerant genotypes against both ChiLCuV and anthracnose diseases were identified which were less frequent in *C. annuum* than other domesticated species and inter-specific derivatives.

Keywords: *Capsicum annuum,* ChiLCuV, Chilli anthracnose, Variability, Divergence.

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Introduction

An understanding of genetic diversity forms the basis of the selection of germplasm to be utilized in the hybridization program. Chilli is considered an often or facultative cross-pollinated crop and the degree of outcrossing at the field level has been reported to range from 7 to 90% (Tanksley, 1984; Singh *et al*., 1994). India is considered to be the secondary center of diversity of chilli, especially of *Capsicum annuum* (Dhaliwal *et al*., 2014), therefore, an ample amount of variability in *C. annuum* exists all over the country (Kumar *et al*., 2006). Despite such variability, the crop fails to attain optimum productivity owing to the use of local unimproved cultivars and susceptibility to several biotic stresses.

In India, anthracnose, a seed-borne disease caused by *Colletotrichum capsici*, was first reported by Sydow (1928) from Coimbatore of Madras Presidency, is an emerging threat in chilli production that spreads under humid conditions, leaving very limited chances for growers to protect the crop (Srideepthi *et al*., 2017). The disease results in dark spots, sunken necrotic tissue with concentric rings of acervuli, including die back in the stem, seedling blight, or damping off (Azad *et al*., 1991) and has reportedly caused marketable yield loss ranging from 50 to 80% in different parts of the world (Sariah, 1994). The accessions of *C. annuum* species are known to be highly susceptible to anthracnose (Yoon *et al*., 2004) and commonly utilized resistant genotypes are non-*C. annuum*, especially *C. baccatum* and *C. chinense* (Bal *et al*., 2024; Lee *et al*.,

2010). A continuous search for new sources of resistance among *C. annuum* types and utilization of these genetic resources in breeding for the development of better varieties/hybrids with higher levels of tolerance against ChiLCuV and anthracnose is necessary.

Genetic divergence is an important criterion to identify potential donors in a hybridization program and proper choice of parents among indigenous materials based on multivariate analysis is essential for the development of recombinant(s) tolerant to both ChiLCuV and chilli anthracnose diseases. An investigation was carried out to determine the breeding potential of chilli genotypes against ChiLCuV and anthracnose diseases through multivariate analysis.

Materials and Methods

Breeding materials and experimental location

Twenty-six advanced breeding lines/varieties/accessions of chilli, collected across India, constituted the plant materials for this study and were screened in the research field of All India Coordinated Research Project on Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India located at 23.5° N latitude and 89° E longitude at an elevation of 9.75 m above the mean sea level.

Cultural practices

The experimental soils were non-saline (EC 0.285 dS/m), sandy-loam in texture, almost neutral in action (pH 6.8), and low in organic carbon (4.2 g/kg), having good drainage facilities. Seedbeds were prepared in sandy loam soil and were 20 cm high and 1.0 m wide. Weathered cow dung @ 4 kg/m^2 was mixed into the beds. Thereafter, beds were drenched with chlorothalonil (2 g) + Carbendazim (1g) to keep away from damping off disease. Seeds, after treatment with Thiram (3 g/kg of seed), were sown on 28 September 2019 at a shallow depth of 5 cm apart and covered thereby with finely sieved well, rotten leaf mold (leaves of which were left to decompose for two years) which acts simultaneously as a soil improver and in preventing soil from drying out. After sowing, beds were covered with straw until germination which normally takes 7 to 10 days and hand watered regularly up to 24 October 2019. Nursery beds were then covered with 200 µm ultraviolet (UV)-stabilized polythene film supported by bamboo poles with open sides to protect seedlings from rain and direct sunlight. Seedlings were hardened by withholding water 4 days before transplanting. Thirty-day-old seedlings were transplanted to the main field on 28 October 2019. Management practices as scheduled for cultivation were followed as per Chattopadhyay *et al*. (2007).

Observations recorded

Observations were recorded from 10 randomly selected plants out of 25 plants in each of three replications for plant height (cm), plant spread (cm), specific leaf weight (g), number of branches per plant, days to 50% flowering, number of fruits per plant, fruit retention (%), days to ripe fruit maturity from anthesis, fruit yield per plant (g), and 15 randomly selected fruits from plants of each replication for fruit length (cm), fruit diameter (cm), average fruit weight (g), number of seeds per fruit, 1000 seed weight (g).

Data on the percent disease index (PDI) of ChiLCuV was determined at 3 stages at an interval of 30 days, starting from 30 DAT and continuing up to 90 days after transplanting. PDI of ChiLCuV was obtained from all 25 plants in replication based on a disease scoring scale (0-9) as per Reddy *et al*. (2001) with slight modifications.

The maximum prevalence of anthracnose disease occurs during the *Kharif* season (July–October) under open field conditions. However, the present research work was conducted during the autumn-winter season when the prevalence of anthracnose disease under open field conditions was very low to assess the severity of anthracnose disease. The severity of anthracnose disease was judged under laboratory conditions. Artificial inoculation of fruits was done to get accurate results. Chilli fruit showing the typical symptoms of fruit rot was collected from different places in West Bengal state. The pathogen was isolated in a potato dextrose agar (PDA) medium using the collected samples. About 15 days old culture of *Colletotrichum capsici* was used for artificial inoculation. Twenty-five red ripe fruits from randomly selected plants of each genotype in each replication were considered for inoculation. The fruit surface was sterilized with 0.1% $HgCl_2$, and then washed in two changes of sterile water. Thereafter, the fruits were pricked with sterile pin bundles. The pinpricked fruits were then dipped in spore suspension (5×10^5 spores/mL) of anthracnose fungus for 5 minutes and then kept for incubation on trays under a humid chamber. The humid chamber was prepared by keeping water in a tray that was placed below the tray with inoculated fruits. The wetted cotton pieces were placed on the trays and were covered with polythene to maintain the relative humidity and incubated at 27 ± l°C thereafter for a week. After the 7th day of inoculation, infected samples were observed for anthracnose lesions. A disease rating scale following Singh *et al*. (1993) was used for the identification of tolerant/susceptible genotypes in chilli.

Statistical Analysis

The analysis of variance was done as per the standardized method described by Panse and Sukhatme (1967). Genotypic and phenotypic coefficients of variation were worked out using the formulae of Burton (1952). Heritability in a broad sense, was calculated as per the formula given by Lush (1949). The genetic advance was estimated following Johnson *et al.* (1955). Phenotypic and genotypic correlation coefficients were computed as per Johnson *et al.* (1956). Direct and

indirect effects of yield on different components and disease severity traits were calculated through path co-efficient analysis as per Dewey and Lu (1959). The D^2 statistic was used to assess genetic divergence between populations (Mahalanobis, 1936). The grouping of populations was done by using Tocher's method as described by Rao (1952). Hierarchical cluster analysis was performed to observe the degree of association according to characteristics expressed in a dendrogram (Ward, 1963). Principal component analysis (PCA) was used to identify the factor dimension of the data and to summarize varietal information in a reduced number of factors for the selection of the best-performing genotype(s). Statistical analyses were with Windostat (ver. 8.0, Indostat Services, Hyderabad, India) and SAS (ver. 9.3, SAS Inc., Cary, NC).

Results and Discussion

PDI values of genotypes for ChiLCuV at different days after transplanting (DAT) varied. Most of the genotypes had comparatively lower PDI values at 30 DAT (Fig. 1). PDI values gradually increased to 60 DAT, and values varied from 3.51 to 66.11%. At 90 DAT, genotypes 'Bidhan Chilli 4' (5.18%), 'BCC 1' (7.55%), 'Chinese Bona' (7.96%) and 'Pant C 1' (10.00%) recorded the lowest disease severity and were considered as 'resistant' to ChiLCuV. In addition, the genotypes 'Dhani Lanka,' 'Akashi,' 'PBC-824', 'PBC-613', and 'Chilli IR-8' were considered to be 'moderately resistant' according to the categorization scheme laid down by Reddy *et al*. (2001). High PDI values were observed in accessions, 'IC-255916', 'IC-383072', 'EC-390029', 'EC-382175' and 'IC-255944' at 90 DAT (Fig. 1). Resistant and moderately resistant genotypes against ChiLCuV may be used under integrated production systems and in developing new resistant genotypes.

Genotypes were screened under artificial conditions to judge their potentiality to combat chilli anthracnose diseases and genotype-dependent responses were observed (Fig. 2). Chilli fruits were inoculated with *Colletotrichum capsici* under laboratory conditions and genotypes 'Bidhan Chilli 4' (1.73%), 'Pant C 1' (2.22%), 'Chinese Bona' (4.53%), and 'Chilli 38-Ragi' (5.60%) exhibited resistant disease reaction as per the categorization scheme laid down by Singh *et al*. (1993). Moderately resistant disease reaction was observed in the genotypes 'Srinagar', 'IC-570408', and 'BCC 1'. In addition, genotypes 'IC-383072', 'EC390029', 'G-4 Ziya Chibli', 'PBC-613', 'G-4', 'EC-382175', 'Akashi Lanka', 'Phule Jyoti Garima', 'IC-255944', 'BCC-25' and 'BCC-30' were considered as susceptible where disease reaction ranged from 25-50%. Disease reaction was more than 50% in the rest of the genotypes as per the categorization laid out by Singh *et al*. (1993).

Analysis of variance indicated genotype was important for genetic variability (Table 1). Very high variance (mean sum of squares for genotypes) was recorded in plant height, plant spread, fruit retention (%), fruit yield per plant, PDI of ChiLCVD (%), and PDI of chilli anthracnose disease (%), indicating a very wide range of diversity concerning these characters (Table 1).

The coefficient of phenotypic and genotypic variation (PCV and GCV, respectively), heritability in a broad sense (h^2_{bs}), and genetic advance as a percent of the mean for the characters varied (Table 2). Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) revealed close resemblance for all the characters except the number of fruits per plant and fruit yield per plant, which indicated the fact that the contribution of these characters towards final phenotypic expression was mainly due to genetic makeup of the genotypes rather than environmental influences and selection alone could be effective based on phenotypic characters. The PCV values were found to be higher than the corresponding GCV values, indicating that there was a significant influence on the growing environment. High GCV and PCV values (>20%) occurred in plant height, plant spread, number of branches per plant, days to 50% flowering, number of fruits per plant, fruit retention (%), fruit length, fruit diameter, average fruit weight, number of seeds per fruit, 1000 seed weight, fruit yield per plant, PDI of ChiLCVD (%) and PDI of chilli anthracnose disease (%) except days to ripe fruit maturity from anthesis and specific leaf weight. The high magnitude of GCV and PCV indicates ample scope for improvement through normal selection. The proportion of GCV to PCV was high (> 90%) for specific leaf weight, days to 50% flowering, fruit retention (%), fruit length, fruit diameter, average fruit weight, number of seeds/fruit, 1000 seed weight, days to ripe fruit maturity from anthesis, fruit yield per plant, PDI of ChiLCuV and chilli anthracnose diseases. Traits with such high proportions are desirable in the selection process as it depicts that the traits are under genetic control rather than the environmental effect. Traits whose expressions are environmentally dependent may not be reliable descriptors for morphological characterization (Pandey *et al*., 2008; Samaee *et al*., 2003). The proportion of genetic contribution to the overall phenotypic expression of most traits was very high. Thus, their use as an important discriminatory variable for chilli classification studies seems relatively reliable.

The genotypic coefficient of variation is the measure to estimate the variability of characters, but GCV alone cannot determine the amount of variation that is heritable. The GCV × selection differential helps in estimating the maximum effectiveness of selection and heritability indicates how closely the goal can be achieved (Singh *et al*., 1968; Singh and Singh, 1985).

Heritability is of interest to plant breeders primarily as a measure of the value of selection for a particular character in various types of progenies and as an index of transmissibility of characters from parent to offspring (Hayes *et al*., 1955). The

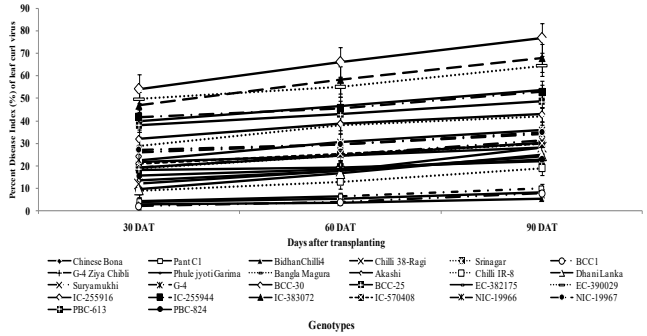

Fig. 1: Disease severity of chilli leaf curl virus (ChiLCuV) at periodic intervals among chilli genotypes

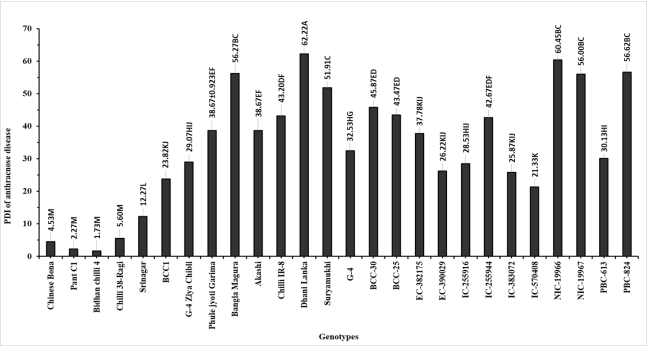

Fig. 2: Disease severity of chilli anthracnose among chilli genotypes under the artificial condition

concept of heritability is important to evaluate the relative magnitude of the effects of genes and environments on total phenotypic variability. For this reason, Burton (1952) stated that genetic variability, along with heritability, should be considered to assess the maximum and accurate effect of selection. High broad sense heritability (60% and above) occurred for all characters under study (Table 2). High heritability for fruit length, fruit diameter, and fruit weight supports the results of Chattopadhyay *et al*. (2011), and the other observations corroborate the findings of previous workers (Vaishnavi *et al*., 2018; Bhutia *et al*., 2015; Hasanuzzaman *et al*., 2012; Rosmania *et al*., 2016) who utilized other genotypes under different growing environments. High heritability indicates that the environmental influence is minimal on characters; the characters can be used for selection.

Genetic advance (GA) is an improvement in the performance of selected lines over the original population. It is not necessarily true that high heritability would always exhibit high genetic advances. For this reason, Johnson *et al*. (1955) stated that heritability in combination with genetic advance would be more reliable for predicting the effects of selection because genetic advance depends on the amount of genetic variability, the magnitude of the masking effect of genetic expression (environmental influence), and intensity of selection. In the present study, GA was very high

Table 1: Analysis of variance (ANOVA) for sixteen quantitative traits in chilli

Source of variation	*Mean sum of square*		
	Replication	*Treatments*	*Error*
Degrees of freedom	*2*	*25*	*50*
Plant height	0.7642	1249.7160**	110.3770
Plant spread	0.6132	1199.1614**	126.8037
Specific leaf weight	0.0004	2.5350**	0.0001
Number of branches per plant	0.0512	9.428205**	0.9712
Days to 50% flowering	0.6282	691.5261**	5.2015
Number of fruits per plant	9.2692	584.9994**	48.6425
Fruit retention	2.5512	8526.1153**	14.9246
Fruit length	0.0857	6.5694**	0.1129
Fruit diameter	0.0004	1.6731**	0.0124
Average fruit weight	0.0002	1.0448**	0.0029
Number of seeds per fruit	9.2692	584.9994**	48.6425
1000 seed weight	0.0008	2.6784**	0.0034
Days to ripe fruit maturity from anthesis	0.3589	157.5533**	2.7856
Fruit yield per plant	6.0823	6036.3140**	207.2829
PDI of ChiLCuV	0.2648	1029.0531**	7.6296
PDI of chilli anthracnose	1.4221	1009.4875**	2.9737

** Significant at 0.05% level of probability

Table 2: Mean, range and estimates of genetic parameters of chilli genotypes

Characters	*Range*	*GCV[a] (%)*	*PCV[b] (%)*	*GCV:PCV[c]*	*h^{2d} (%) in b.s.*	*Genetic advance as (%) of mean*
Plant height	47.83-117.13	26.59	30.21	88.02	77.50	61.79
Plant spread	27.57-92.17	31.06	36.16	85.91	73.80	70.46
Specific leaf weight	3.20-6.59	19.32	19.32	99.98	99.00	51.00
Number of branches per plant	4.67-11.33	23.17	26.87	86.24	74.40	52.77
Days to 50% flowering	21.33-74.33	30.56	30.91	98.88	97.80	79.78
Number of fruits per plant	26.67-77.00	28.85	32.53	88.66	78.60	67.53
Fruit retention	28.00-252.33	56.28	56.43	99.74	99.50	148.21
Fruit length	3.70-9.47	21.28	21.83	97.48	95.00	54.77
Fruit diameter	0.45-2.75	47.80	48.34	98.89	97.80	124.81
Average fruit weight	0.64-2.88	37.85	38.02	99.58	99.20	99.53
Number of seeds per fruit	28.33-86.67	27.31	27.52	99.25	98.50	71.58
1000 seed weight	0.84-4.86	25.31	25.36	99.80	99.60	66.69
Days to ripe fruit maturity from anthesis	33.33-57.33	16.26	16.69	97.4	94.90	41.81
Fruit yield per plant	30.50-222.15	59.69	62.79	95.05	90.40	149.81
PDI[e] of ChiLCuV[f]	5.18-76.78	54.97	55.58	98.89	97.80	143.52
PDI of chilli anthracnose	1.73-62.22	54.26	54.50	99.56	99.10	142.62

[a]GCV= Genotypic Coefficient of variation.
[b]PCV= Phenotypic Coefficient of Variation
[c]GCV:PCV (%)= ratio of the genotypic coefficient of variation and phenotypic coefficient of variation.
[d]h^2 in b.s. = Heritability estimate in broad sense
[e]PDI = Percent Disease Index
[f]ChiLCuV = Chilli Leaf Curl Virus

(>100 %) for the fruit retention percentage, fruit diameter, fruit yield per plant, PDI of ChiLCVD, and PDI of chilli anthracnose diseases (Table 2). High GA (>20%) was also recorded for the characters plant height, plant spread, specific leaf weight, number of branches per plant, days to 50% flowering, number of fruits per plant, fruit length, average fruit weight, number of seeds per fruit, 1000 seed weight and days to ripe fruit maturity from anthesis.

High genetic advances for these traits have been previously reported (Kranthirekha *et al*., 2016; Elahi *et al*., 2017; Yogeshkumar *et al*., 2018; Lakshmidevamma *et al*., 2021) using dissimilar genotypes and in different environments. Heritability in combination with a substantial amount of GA would be more reliable than heritability alone for predicting the effect of selection in segregating generations (Johnson *et al*., 1955). These two genetic parameters can together substantiate the amount of genetic progress possible through normal selection. High heritability coupled with high estimates of GCV and GA for most traits under study offers opportunities for selection and indicates the presence of additive gene action, which would make the selection very effective (Panse, 1957).

Genotypic and phenotypic correlations among quantitative characters varied (Table 3). Most correlation coefficients at the genotypic level were greater than the corresponding phenotypic ones. The higher values of genotypic than phenotypic correlation indicated that the genotypic effects were more important than environmental factors. In the presence of high environmental influence on the expression of characters, there is the possibility of overestimation of the genotypic correlation coefficient. Most characters exhibited significantly positive genotypic and phenotypic correlations with fruit yield per plant. PDI of ChiLCuV and chilli anthracnose disease were negatively correlated with fruit yield per plant. This indicated that a lower incidence of diseases helped to improve fruit yield per plant. Direct and indirect effects at the phenotypic level on fruit yield per plant varied (Table 3). Average fruit weight and number of fruits per plant had a positive, direct effect on fruit yield per plant, likely due to the positive association with fruit yield per plant. The direct effects of other characters were negligible. Direct selection could be beneficial for yield improvement since the number of fruits per plant and fruit weight exhibited significant, positive correlations with fruit yield per plant. High positive, direct effects of the number of fruits per plant and fruit weight on fruit yield per plant were obtained with other genotypes and environmental conditions by others (Naik *et al*., 2010;

Table 3: Phenotypic and genotypic correlations among different characters of chilli and their direct effects on fruit yield per plant

Character	*Genotypic correlation with fruit yield per plant*	*Phenotypic correlation with fruit yield per plant*	*Direct effects on fruit yield/ plant at phenotypic level*[a]
Plant height	0.719**	0.472**	0.102
Plant spread	0.618**	0.647**	0.037
Specific leaf weight	0.474**	0.454**	-0.128
Number of branches per plant	0.554**	0.571**	0.030
Days to 50% flowering	-0.727**	-0.723**	-0.108
Number of fruits per plant	-0.329**	-0.360**	0.010
Fruit retention	0.742**	0.686**	0.108
Fruit length	0.450**	0.473**	-0.120
Fruit diameter	0.466**	0.474**	0.011
Average fruit weight	0.715**	0.704**	-0.019
Number of seeds per fruit	0.291**	0.263*	-0.058
1000 seed weight	0.709**	0.735**	0.574
Days to ripe fruit maturity from anthesis	0.795**	0.779**	0.674
PDI[b] of ChiLCuV[c]	-0.778**	-0.700**	0.073
PDI of chilli anthracnose	-0.557**	-0.547**	0.021

*,** Significant at $p \leq 0.05$ or $p \leq 0.01$, respectively
[a]Residual effect-0.1243
[b]PDI = Percent disease index, [c]ChiLCuV = Chilli leaf curl virus

Table 4: Cluster classification of chilli genotypes

Cluster	Name of the genotype with source of collection
Cluster I (4)[a]	Bangla Magura (W.B.[b]), NIC-19966 (NBPGR[f]), G-4 Ziya Chibli (W.B.), EC-382175 (NBPGR)
Cluster II (4)	Phule Jyoti Garima (MPKV[g]), PBC-613 (NBPGR), PBC-824 (NBPGR), IC-570408 (NBPGR)
Cluster III (3)	Dhani Lanka ((W.B.), Suryamukhi (W.B.), NIC-19967 (NBPGR)
Cluster IV (4)	EC390029 (NBPGR), IC-255944 (NBPGR), IC-255916 (NBPGR), IC-383072 (NBPGR)
Cluster V (3)	BCC-30 (BCKV[h]), BCC-25 (W.B.), G-4 (A.P.[c])
Cluster VI (8)	Chilli 38-Ragi (W.B.), Srinagar (J & K[d]), Pant C 1 (U.K.[e]), Chinese Bona (W.B.), Akashi Lanka (W.B.), Chilli IR-8 (W.B.), Bidhan Chilli 4 (BCKV), BCC1 (BCKV)

[a]Value in the parantheses after cluster number indicate number of genotypes.
[b]W.B. = West Bengal
[c]A.P. = Andhra Pradesh
[d]J & K = Jammu and Kashmir
[e]U.K. = Uttarakhand
[f]NBPGR = National Bureau of Plant Genetic Resources
[g]MPKV = Mahatma Phule Krishi Vidyapeeth
[h]BCKV = Bidhan Chandra Krishi Viswavidyalaya

Kumari *et al*., 2010; Thakur *et al*., 2019). The residual effect was very low indicating inclusion of maximum fruit yield per plant influenced characters in the analysis. Based on the degree of divergence (D^2 values), genotypes could be meaningfully grouped into 6 clusters (Table 4). Cluster VI had a maximum of 8 genotypes, followed by clusters I, II, and IV with 4 genotypes each; clusters III and V had 3 genotypes each. The grouping pattern of genotypes was random, indicating geographical diversity and genetic divergence were unrelated. Genotypes grouped from different heterogeneous geographic regions in one cluster could result from a free exchange of breeding material from place to place either by farmers or breeders of different regions. The absence of a relationship between genetic diversity and geographical distance depicts that forces other than geographical origin, such as genetic drift, exchange of genetic stock, spontaneous mutation, and natural and artificial selection, are responsible for genetic diversity. The

selection of genotypes for the hybridization program should be based on genetic divergence rather than geographic divergence. Environmental influence on the composition of clusters occurs in chilli (Krishnamurthy *et al*., 2013; Yatung *et al*., 2014; Bhutia *et al*., 2017).

The clustering pattern indicated that inter-cluster distance was higher than intra-cluster distance indicating high genetic diversity among genotypes taken under study (Table 5). Lower intra-cluster divergence indicated homogeneity among genotypes that were clustered together. The intra- and inter-cluster distance among the genotypes indicated cluster VI had the highest intra-cluster distance indicating genotypes in this cluster are diverse. At the inter-cluster level, the maximum inter-cluster value was in between cluster II and VI, followed by between cluster I, and V, indicating genotypes in these clusters had maximum divergence. Intermating between genotypes included in clusters VI, V, II and I would be expected to give transgressive segregates in advanced generations (Kalloo *et al*., 1980).

The top characters which contributed maximum towards divergence were PDI of ChiLCuV followed by PDI of chilli anthracnose disease and plant height (Table 6). Such characters may be used in selecting genetically diverse parents for hybridization to exploit maximum heterosis or to accomplish selection coherently in segregating generations.

Cluster means of genotypes (Table 6) indicated mean values of clusters varied in magnitude for all 16 characters. Cluster VI showed the highest number of branches per plant, fruit retention percentage, fruit length, fruit diameter, 1000 seed weight, average fruit weight, and fruit yield per plant, followed by cluster V for maximum plant height, plant spread, specific leaf weight, number of seeds per fruit, number of fruits per plant. Besides, cluster means for PDI of ChiLCuV and chilli anthracnose disease, days to 50% flowering and days to ripe fruit maturity from anthesis were found lowest in cluster VI. Genotypes belonging to cluster VI could be useful sources of genes for imparting tolerance against viral and fungal diseases by improving productivity with early plant types that can fit in any cropping system. Intercrossing among genotypes having outstanding mean performances can be used for improvement in chilli (Yogeshkumar *et al*., 2018; Lakshmidevamma *et al*., 2021). High genetic diversity among genotypes exists along with strong relationships among them as indicated in the dendrogram (Fig. 3).

Differences in allele frequency (y) of parents and dominance effect (D) at various loci is the dependent factors for the expression of heterosis over mid-parents (H), i.e., $H = Dy^2$ (Falconer, 1981). A certain level of genetic diversity and degree of dominance are important factors for the expression of heterosis. A hybridization program involving highly divergent parents may produce transgressive segregates. There lies an optimal level of diversity beyond which heterosis may either decrease or might not increase due to unfavorable interaction of co-adopted gene complexes or physiological incompatibility (Dhillon *et al*., 2004).

The PCA components with eigenvalues exceeding 1.0 explained 100% of the total variance (Table 7). The characters viz., PDI of ChiLCuV, PDI of chilli anthracnose disease and, plant height explained almost 100% contribution towards divergence and variable loadings for components PC_1 (PDI of ChiLCuV), PC_2 (PDI of chilli anthracnose disease), PC_3 (plant height) were shown in Table 8. The first component

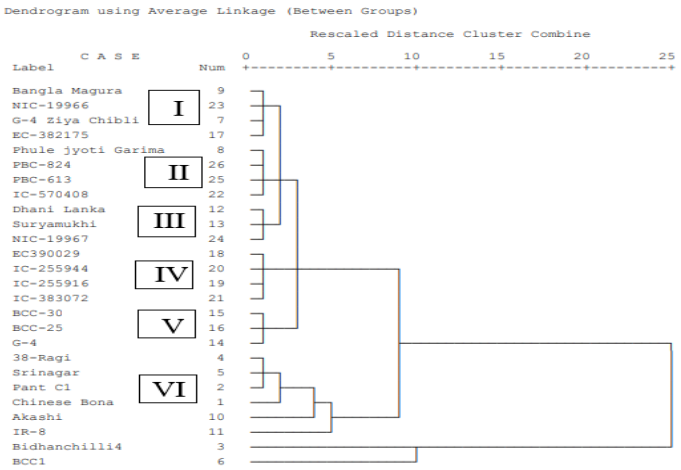

Fig. 3: Dendrogram of genotypes of chilli following Ward's method. Genotypes are in the leftmost column. The horizontal axis of the dendrogram represents the distance or dissimilarity between clusters. The vertical axis represents the objects and clusters. Each joining (fusion) of two clusters is represented on the graph by the splitting of a horizontal line into two. The horizontal position of the split, shown by the short vertical bar, gives the distance (dissimilarity) between the two clusters. Clusters are identified by Roman numerals. Genotypes placed in clusters based on 11 quantitative traits (Plant height, plant spread, specific leaf weight, number of branches per plant, days to 50% flowering, number of fruits per plant, fruit retention, fruit length, fruit diameter, average fruit weight, number of seeds per fruit, 1000 seed weight, days to ripe fruit maturity from anthesis, fruit yield per plant, PDI of ChiLCuV, PDI of Chilli anthracnose) all taken together.

Table 5: Inter and intra-cluster distances of chilli genotypes

Clusters	*I*	*II*	*III*	*IV*	*V*	*VI*
I	10730.050	38274.680	77275.630	85834.030	343362.600	232599.700
II		10968.067	161242.600	132961.900	324543.600	470168.000
III			13178.980	35155.390	134511.100	81509.670
IV				15284.670	121794.800	55772.160
V					15676.100	27353.170
VI						23246.430

[a]Bold diagonal values indicate intra-cluster distance; the remainder of values indicate the inter-cluster distances

Table 6: Cluster means of chilli genotypes

Characters	*Cluster I*	*Cluster II*	*Cluster III*	*Cluster IV*	*Cluster V*	*Cluster VI*	*%contribution towards divergence*
Plant height	51.45	66.91	78.74	66.98	95.66	94.74	10.65
Plant Spread	47.74	49.68	63.48	59.60	92.16	77.13	0.75
Specific leaf weight	4.16	3.58	4.82	5.04	6.59	5.91	0.45
Number of branches per plant	6.91	6.66	7.26	7.33	7.33	8.06	2.50
Days to 50% flowering	55.75	56.55	46.86	51.80	55.66	35.06	0.79
Number of fruits per plant	40.08	42.00	43.06	43.53	73.00	57.33	4.75
Fruit retention	64.66	58.05	95.46	84.06	84.33	174.26	0.50
Fruit length	5.33	6.58	7.47	6.70	7.30	8.03	2.29
Fruit diameter	1.57	1.24	1.49	1.38	1.93	2.07	1.36
Average fruit weight	1.05	1.25	2.22	1.24	1.16	2.04	0.21
Number of seeds per fruit	36.75	54.33	59.26	51.06	76.33	73.33	0.50
1000 seed weight	3.76	3.78	3.32	3.17	4.16	4.51	0.20
Days to ripe fruit maturity from anthesis	44.41	46.22	44.46	46.86	43.66	38.60	0.35
Fruit yield per plant	41.19	49.41	102.09	54.281	85.03	118.34	3.38
PDI[a] of ChiLCuV[b]	55.99	36.20	24.51	42.162	24.26	14.76	50.50
PDI of chilli anthracnose	34.02	44.62	29.11	40.711	38.66	17.21	20.82

[a]PDI = Percent Disease Index
[b]ChiLCuV = Chilli Leaf Curl Virus

(PC1) explained 52.67% of the total accounted for variance in which a decrease in PDI of ChiLCuV was associated with a decrease in PDI of chilli anthracnose disease but will eventually increase plant height. The second component (PC_2) explained 20.96% of the total accounted for variance in which a decrease in PDI of Chilli anthracnose disease was associated with an increase in PDI of ChiLCuV and plant height. There are no such guidelines for determining the importance of a trait coefficient for each principal component. Johnson and Wichern (1998) regard a coefficient greater than half of the coefficient, divided by the square root of the standard deviation of the eigenvalue of the respective principal component, to represent a significant difference.

The dendrogram was formed following Ward (1963) using squared Euclidean distance. There exists high diversity among chilli genotypes exhibiting strong relationships among genotypes (Fig. 3). All 26 genotypes were grouped into 6 distinct clusters based on average linkage between 2 clusters representing characteristic similarities and dissimilarities. The higher the rescaled distance joining a genotype, the higher the dissimilarities in characteristic features and vice versa. Genotypes that are close are perceived for their similarity in PCA; but the genotypes which are far apart are considered more diverse (Fig. 4). Genotypes 'Chinese Bona', 'Pant C 1', 'Bidhan Chilli 4', 'Chilli 38-Ragi', 'Srinagar', and 'BCC 1' were quantitatively dissimilar from others. The remainder of the genotypes had similar features,

Table 7: Results of principal component analysis (PCA) for quantitative characters contributing to divergence

Principal component (PC)	*Eigenvalue*	*%Variance*	*%Cumulative Variance*
PC1	3.16030215	52.67	52.67
PC2	1.25770058	20.96	73.63
PC3	0.67213122	11.20	84.84

[a]Eigenvalues and variance accounted for (%) by PCA based on the correlation matrix

Table 8: Contribution of diverse traits in the principal components of chilli

Variables	PC_1 [a]	PC_2	PC_3
Factor loadings due to PCs with eigenvalues greater than 1			
PDI[a] of ChiLCuV[b]	-0.445696	0.419436	-0.046058
PDI of chilli anthracnose	-0.360723	-0.321216	0.768103
Plant height	0.464195	0.186041	0.436577

[a]PC_{1-3} = principal components 1-3
[a]PDI = Percent disease index
[b]ChiLCuV = Chilli Leaf Curl Virus

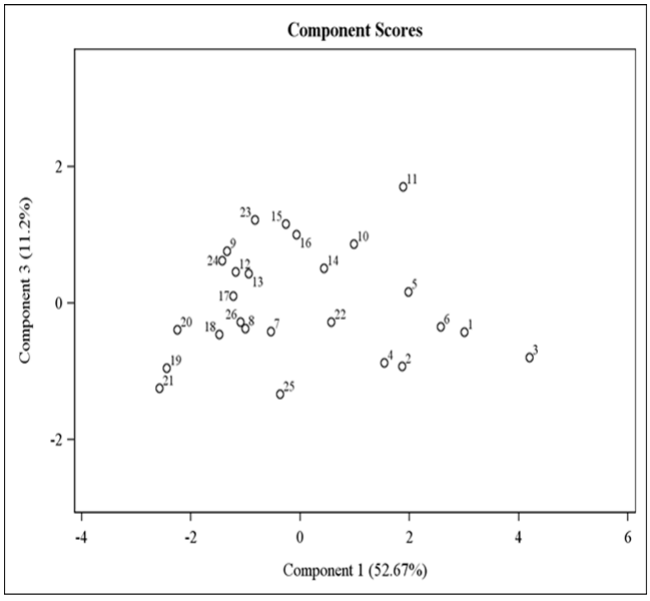

Fig. 4: Scatter diagram of regression factor scores for the first and third components as determined by principal component analysis. Points in the diagram closest to the intersection of 0 on the X- and Y-axes indicate similarity. Outliners on the X-axis, i.e. 1 = Chinese Bona, 2 = Pant C 1, 3 = Bidhan Chilli 4, 4 = Chilli 38-Ragi, 5 = Srinagar and 6 = BCC 1, indicate diversity. Numbers correspond to the names of the genotypes in Fig. 3.

forming a separate cluster. From PC_1 vs. PC_3 plot, selection may be refined considering all 3 components, with 'Bidhan Chilli 4' being the best performing genotype having high tolerance to both ChiLCuV and chilli anthracnose diseases and an optimum combination of all variables followed by 'Pant C 1', 'Chinese Bona' can be used as improved genetic material for breeding against ChiLCuV and chilli anthracnose diseases, respectively.

This was one of the few attempts to identify potential donors among *C. annuum* L. genotypes for developing multiple disease resistance (ChiLCuV and anthracnose) having preferred economic traits. Genetic divergence and geographic diversity were unrelated in chilli concerning the occurrence of ChiLCuV and anthracnose diseases. Based on D^2 statistics, PCA, yield potentiality, and reaction to both ChiLCuV and chilli anthracnose diseases, the genotypes, 'Bidhan Chilli 4', 'Pant C 1', and 'Chinese Bona' were identified as potential donors. The linkage between these two diseases in identified genotypes will help breeders to develop multiple disease-resistant varieties/hybrids to avoid the indiscriminate use of pesticides.

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